Supplemental Data

Mesenchymal Stem Cells Reduce Corneal Fibrosis and Inflammation via Extracellular Vesicle-Mediated Delivery of miRNA

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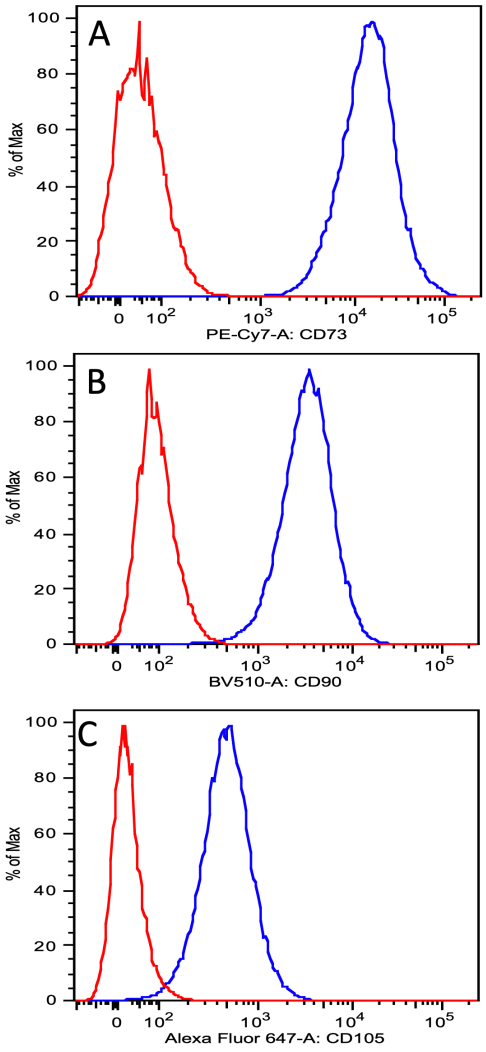
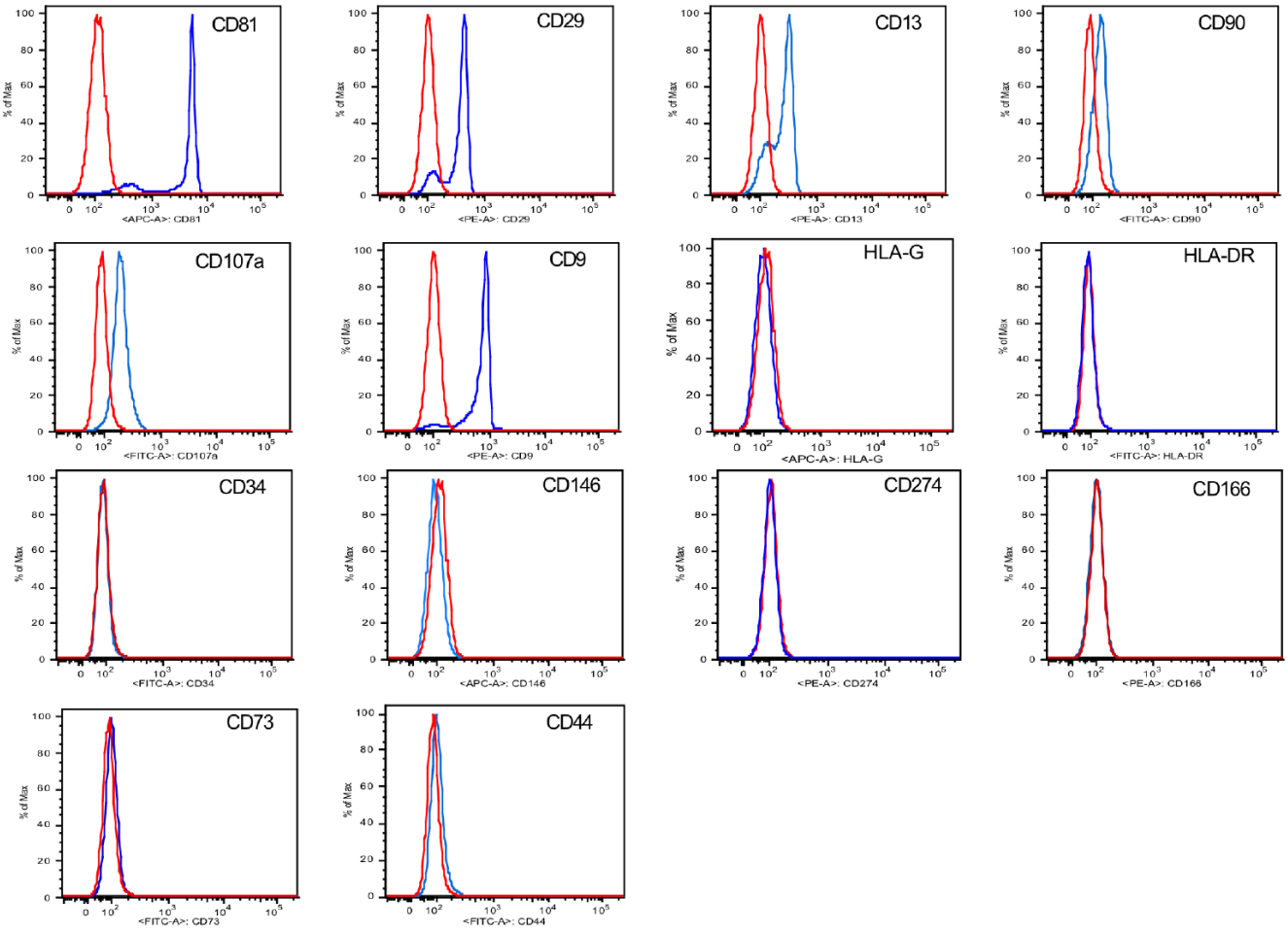
Figure S1



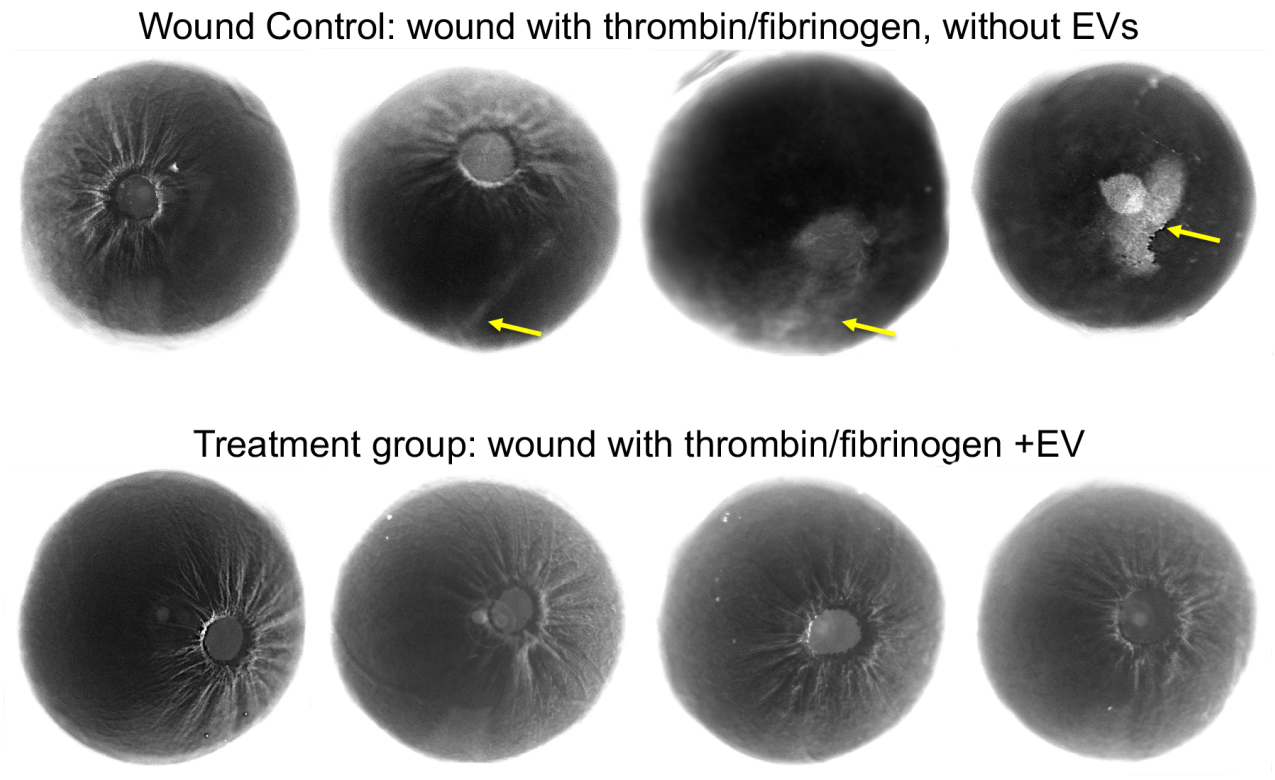
Supplemental Fig S1. MSC marker expression by CSSC. Subconfluent CSSC cells (line HC461) were stained with (**A**) CD73 (**B**) CD90, and (**C**) CD105, and analyzed by flow-cytometry. Blue trace shows a histogram of cells incubated with the indicated antibody. Red trace shows cells incubated in parallel with a non-specific (isotype) antibody labeled with the same fluor.

Figure S2



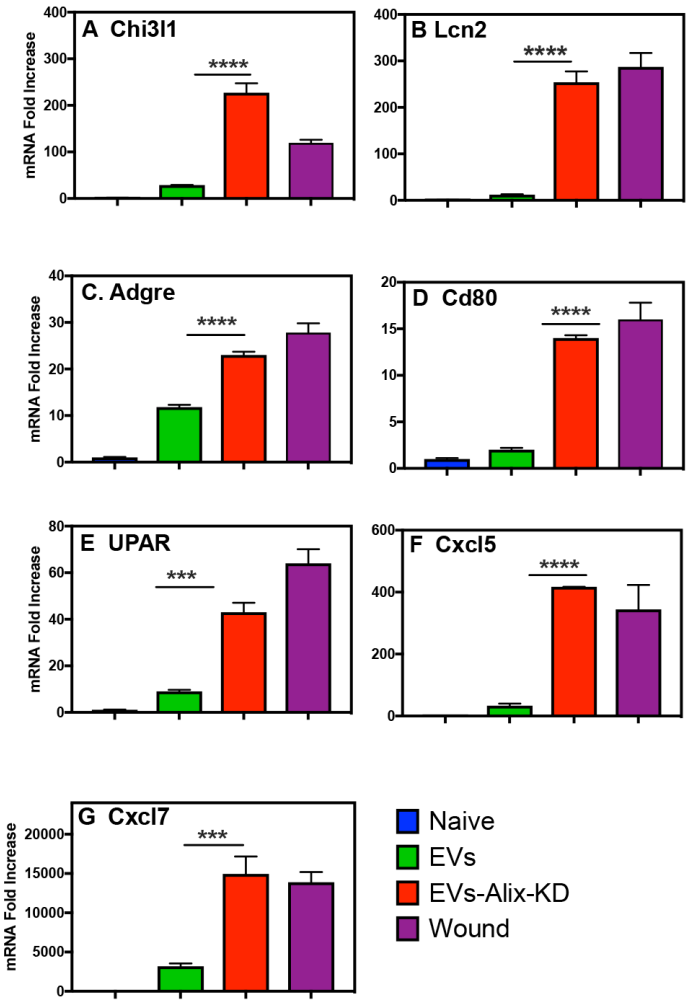
Supplemental Fig S2. Identification of cell surface proteins on purified EVs from CSSC. CSSC EVs bound to anti-CD63 beads were probed with fluorescent antibodies to diverse cell surface proteins and then separated by flow cytometry as described under Methods. Graphs show histogram of fluorescence intensity (x-axis) vs bead count (y-axis). The figure illustrates all of the proteins found to be positive (CD81, CD29, CD13, CD90, CD107a, CD9) as well as some of the negative proteins.

Figure S3



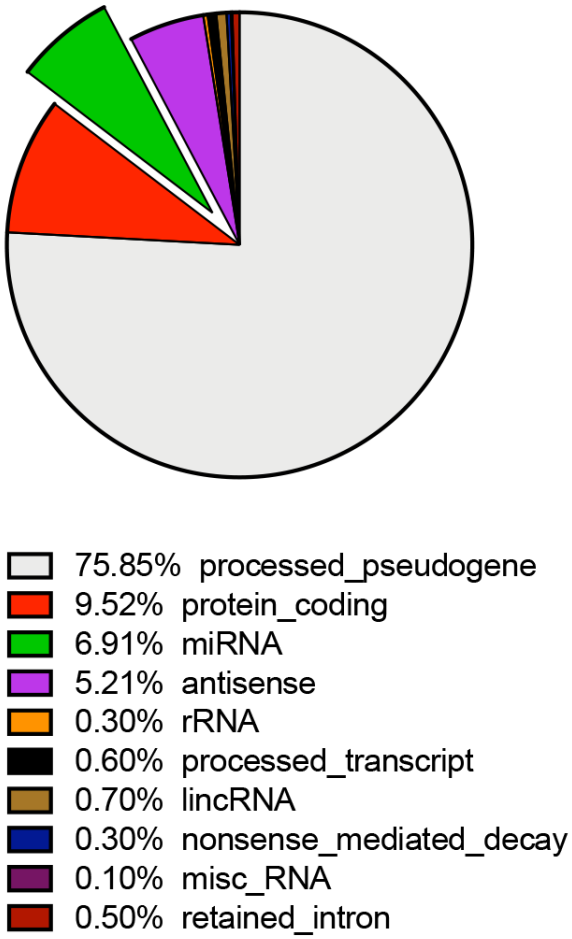
Supplemental Fig S3. Visual scoring of corneal scars. Ex-vivo indirect lighting was used to reveal scarred areas of mouse corneas 14 days after wounding. Normal corneal tissue is not visible in this lighting, but scarring appears as a white haze obscuring the iris (arrows). 50-70% of wounded corneas showed visible corneal scarring when treated with vehicle (fibrinogen/thrombin) whereas none of the eyes treated with EVs (+EVs) had visible scars. Light color of the pupil area is caused by lens opacity not corneal scarring.

Figure S4



Supplemental Fig S4. Expression of inflammatory genes in wounded corneas. Three days after wounding, expression of mouse mRNA for known inflammatory genes was compared in non-wounded (Naïve, blue) corneas, wounded corneas (Wound, purple), and for wounded corneas treated with EVs (EVs, green) or treated with EVs from cells transfected with siRNA against Alix (EVs- Alix-KD, Red). Expression values are given relative to Naïve cornea =1. Statistical comparisons were by t-test (n=3). For each gene, expression in wounded corneas was increased ($p< 0.001$) and corneas treated with EVs had reduced expression compared to wounded corneas ($p<0.01$). In each case, EVs from Alix knockdown cells did not suppress gene expression to the extent of control EVs (***, $p<0.001$, **** ,$p< 0.0001$). In the case of Lcn2, Cd80, Cxcl5, Cxcl7, Alix-KD EVs induced no suppression of gene expression compared to the untreated wounds ($p>0.05$).

Supplemental Fig S5.



Supplemental Fig S5 Types of RNA in EVs from CSSC. Sequence of RNA isolated from purified EVs were analyzed for RNA using RNAseq as described in Methods. The graph shows distribution of different RNA types identified in a typical sample. The exploded section (green) represents the proportion of RNA with sequences identified as micro RNA.

Supplemental Table S1. miRNA Content of CSSC and HEK293T EVs[1]

miRNA	CSSC	HEK	Ratio	p value
hsa-miR-4454/7975	879	409	2.2	0.063
hsa-miR-630	269	1	193	0.023
hsa-miR-612	179	5	33	0.000
hsa-miR-363-3p	177	2	85	0.000
hsa-miR-644a	136	2	56	0.000
hsa-miR-125b-5p	121	85	1.4	0.270
hsa-miR-378h	107	1	77	0.001
hsa-miR-411-5p	104	4	26	0.000
hsa-miR-598-3p	100	11	9.2	0.000
hsa-miR-107	99	89	1.1	0.282
hsa-miR-199a/b-3p	98	3	30	0.015
hsa-miR-1286	94	2	41	0.000
hsa-miR-212-3p	88	2	56	0.000
hsa-miR-196a-5p	84	52	1.6	0.103
hsa-miR-100-5p	83	3	32	0.003
hsa-miR-379-5p	77	3	26	0.002
hsa-miR-514b-5p	77	1	55	0.007
hsa-miR-495-3p	76	1	62	0.000
hsa-miR-498	74	3	24	0.002
hsa-miR-376a-3p	72	2	34	0.000
hsa-miR-155-5p	72	6	13	0.000
hsa-miR-29a-3p	69	42	1.6	0.149
hsa-miR-320e	69	3	25	0.002
hsa-miR-613	68	1	56	0.000
hsa-miR-1261	68	1	131	0.000
hsa-miR-549a	66	1	95	0.000
hsa-miR-548q	63	2	26	0.008
hsa-miR-6721-5p	63	0	182	0.001

[1]miRNA content of EVs isolated from 4 CSSC cell lines and from HEK293T was determined using a Nanostring CSO-MIR3-12 array as described under Methods.

>200 miRNA species were detected in CSSC lines. Expression levels are presented as per cent of the total miRNA measured in each sample x 100. Mean expression of the 30 most abundant miRNA species in EV from 4 CSSC lines is shown. Abundance of miRNA in HEK 293T EVs is listed for comparison. All but 5 miRNAs from CSSC EVs (shown in red) were significantly more abundant than those in HEK293T EVs based on p values calculated as single-sample t-test. Rows listing two species of miRNA used probes which did not distinguish the two.